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THE USE OF LACTOSE-BILE MEDIUM IN WATER ANALYSIS.

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THE determination of intestinal bacteria as represented by *Bacillus coli* gives, when properly interpreted, a most valuable index of the quality of a water from a sanitary standpoint. Unfortunately the presumptive test with Smith solution, owing to the interference of other growths of bacteria has often proved to be misleading, especially in badly polluted waters.

In a paper previously published,¹ entitled "A New Solution for the Presumptive Test for *Bacillus coli*," the author has described numerous experiments on the selective inhibiting effect upon the growth of bacteria by the various salts and their constituents found in the bile. By the use of bile media most species of bacteria other than *B. coli* are killed or restrained, thus allowing of the free action of the intestinal germs. The tests show that the cholic acid radical of the bile is the effective agent. This conclusion is important in that it admits of the use of sodium glycocholate as an inhibiting salt. In ox bile, which is the material most available, nearly all of the bile salt present is in the form of glycocholate.

The experiments also show that good results are obtained only when an amount of bile salt is used equal to that present in undiluted ox bile but that further concentration is unnecessary. Liquid ox bile filtered after sterilization contains about 110 grams of solid matter per liter, of which about 90 grams consist of bile salts. This is an amount far in excess of that previously employed as a restraining agent but a lesser amount gives less efficient results. The liquid also contains albuminous matter equal in food value to the meat extract employed in the usual media.

An undiluted bile solution sterilized when freshly drawn may be

¹ *Biological Studies by the Pupils of William Thompson Sedgwick*, University of Chicago Press, 1906.

kept in stock until ready for use. It is then decanted or filtered and 1 per cent of lactose, previously dissolved in a small amount of water, is thoroughly mixed with the bile, after which it is tubed and sterilized in an autoclave for 30 minutes at 15 pounds pressure. The fermentation tubes employed are 140 mm. long and 15 mm. in diameter, having an elongated bulb 38 mm. in its shortest diameter. This admits of the addition of 10 c.c. of the water to be tested without too great dilution of the medium.

A large number of market samples of evaporated or inspissated bile were tested, but they were all found to be very acid, and even when neutralized did not give proper results. It has since been established by experiment that if the bile is sterilized in an autoclave when freshly drawn and the filtered liquid evaporated to dryness that this product when kept perfectly dry will remain practically neutral and can be preserved indefinitely. A combination of 110 grams of the solid bile and 10 grams of lactose dissolved in a liter of distilled water will give the same results as the medium made from fresh liquid bile.

It has been shown by extensive tables given in the article previously referred to that negative results for *B. coli* have been often found by the use of Smith solution when there was no question that the germ was present but had been overgrown in the medium and crowded out by other bacteria. On the other hand, experiments recently performed show that apparent positive tests for *B. coli* may be obtained when a mixture of gas producing bacilli other than *B. coli* are added to Smith solution.

The results so far obtained with bile lactose medium point strongly to the fact that no gas producer or mixture of gas producing bacteria will give results as high as 25 per cent of gas except *B. coli*, even when three days' incubation is employed. Attenuated *B. coli* sometimes requires three days for its development to that point. The necessity for the absorption test is therefore eliminated.

In testing a water for *B. coli* dilutions of 0.1, 1.0, and 10 c.c. are planted in the fermentation tubes and incubated at 37.5° C for 48 hours. All tubes giving over 25 per cent of gas are considered to contain *B. coli*. Those having any considerable amount of gas but still under 25 per cent are returned to the incubator for 24 hours longer.

Attenuated *B. coli*, as before stated, will sometimes require 72 hours to give the positive test.

Underground waters not exposed to surface influences and properly filtered waters should not give positive tests in any dilution. Surface springs, open dug wells, and pure surface waters may give positive tests in the 10 c.c. dilution, but in no less quantities. Moderately contaminated waters and those exposed to surface drainage containing small quantities of the excreta of the lower warm-blooded animals may give positive tests in the 1 and in the 10 c.c. dilutions. Such waters are to be looked upon with suspicion. Contaminated waters and those having a large amount of surface wash will give positive tests in the $\frac{1}{10}$, 1, and 10 c.c. dilutions. For grossly polluted waters and sewages higher dilutions may be employed, as in testing sewages and sewage effluents from filters.

At Mt. Prospect Laboratory about 5,000 samples of waters of various degrees of purity and from hundreds of different sources have been tested by means of lactose-bile media, and the results have always agreed with the judgments formed by the complete analysis of the water as well as by careful sanitary inspections of the individual sources of supply. In many instances contamination has been found to exist which would not otherwise have been detected.

The use of this medium gives an exact gauge of the purification brought about by systems of filtration, and in the New York City, Department of Water Supply, Gas and Electricity; six larges and filter beds and two mechanical filter plants, under the author's charge in the Borough of Brooklyn, are daily tested by this method as well as for quantitative bacterial removal.

CONCLUSIONS.—The bile medium previously employed has contained too little bile salt to be effective as a selective inhibiting agent.

The use of the lactose-bile medium herein described for the determination of *B. coli* prevents negative tests from the overgrowth of other bacteria as well as positive tests from mixtures of other gas formers.

By the employment of this new medium more definite results on the sanitary quality of a water may be obtained than by any other test at present in use. The medium is also especially valuable in the testing of filter plants.